APPENDIX-IV-A

Format for Outline (Synopsis) of Proposed Research Work
(To be supplied in four copies)
(Reference Ord. 124.11)

1. Name of the Scholar **(In English)** **Nitesh Raj Keer**

(In Hindi) नितेश राज कीर

2. Title of Research Work **(In English)** **TAXONOMICAL STUDY OF FAMILY ASTERACEAE IN SOUTHERN RAJASTHAN.**

(In Hindi) दक्षिणी राजस्थान में एस्टरेसी कुल का वर्गीकीय अध्ययन।

3. Location

a. Institution / Department where the work to be done

Department of Botany
M. L.V. Government College Bhilwara.
M.D.S. University, Ajmer (Rajasthan)

b. Geographical Area of Investigation, if any

The study area covers eight districts namely Banswara, Bhilwara, Chittorgarh, Dungarpur, Pratapgarh, Rajasamand, Sirohi and Udaipur of Rajasthan. This lies between 23° 11'-25° 97' N latitude to 70° 16'-75 49' E longitudes and covers an area of sq 57301 km.

4. Introduction Importance of Proposed Investigation, Objectives

Introduction

Rajasthan is one of the largest states of India, covering 3,42,239 sq km. and 11% of the total area of the country. It has a unique natural region, located in the western part of the India. It is known to have unique floral diversity in the Asia. The main geographic features of the Rajasthan are the Thar desert and the aravali range, which runs through the state from south west to north east, almost from one end to other, for more than 850 km. Mount Abu lies at the

south west end of the range, separated from the main ranges by west banas river, although a series of broken ridges continues into Haryana in the direction of Delhi.

Importance of Proposed Investigation

Rajasthan is rich in biodiversity due to its variable climatic and geographical conditions. By floral study of Rajasthan it is found that family Asteraceae of flowering plant has a major contribution in biodiversity. South Rajasthan has a humid climate and less desert effect and has more water resources that favor the rich floral components. The family Asteraceae includes a great diversity of species, including annuals, perennials, stem succulents, vines, shrubs and trees. It is well naturalized family throughout the world. Family Asteraceae is a major family of medicinal and ornamental plants.

Taxonomist as well as morphologist worked best on different regions of Rajasthan on a big aspect of total flora of Rajasthan and gave a clue to my proposed research topic Taxonomical Study of family Asteraceae in Southern Rajasthan.

Other taxonomist used morphological analysis for taxonomical purpose for family Asteraceae. Family Asteraceae have unique feature of identification as compared to other families. Asteraceae have pappus, trichome, cypsela that are now being used to compare species. These structures can be studied by various types of microscopes so this approach will be new in taxonomical analysis of family Asteraceae in Southern Rajasthan. This study will be done in micro morphological aspect by light microscopes.

Keeping the above scenario in view, present investigation is undertaken to fulfill the following

Objectives

1. The present study has taken up to survey of Southern Rajasthan in different seasons, collection of herbarium specimens and identification.
2. Provide means of identification of family Asteraceae up to intra specific level through keys, diagnostic description, illustrations and photographs.
3. Determine depleting species of family Asteraceae as per IUCN standard.
4. Determine factors responsible for the depletion of plant species and strategies for the conservation of species and habitat.
5. To study systematic and micro-morphological features such as epidermal, stomatal and trichomes of family Asteraceae for taxonomical purposes.

6. To study ecophysiological aspects such as photosynthetic pigments and membrane stability, foliar mineral components, proline, sugars, amino acids, proteins and phenols of the selected taxa of family Asteraceae to evaluate their adaptive strategies under the prevalent harsher climatic condition of this region.
7. To determine the ethno botanical value of family Asteraceae in area.

5. Review (Work Already Done on the Subject of Proposed Research Study.)

The floral component of Rajasthan has been studied by several workers, Bhandari (1978), Sharma and Tiagi (1979), Shetty and Pandey (1983), Singh (1983), Shetty and Singh (1987-1993), Prasad *et al.*(1996), Tiagi and Aery (2007), Meena and Yadav (2008), Yadav and Meena (2009), Meena and Yadav (2010), Meena (2010), Yadav and Meena (2011,2011), Meena (2014). Earlier works on systematic enumeration of South East Rajasthan especially of Kota district have been contributed by Majumdar (1971, 1976 and 1980) in form of Synoptic flora of Kota division. Sharma and Tiagi (1979), Sharma (2002), Sharma and Siringi (1986) are also have made significant contributions. Flora of Rajasthan by N.K. Sharma, Floristic studies on Hadoti region (2002) provides a vast account of angiosperms.

The Compositae (Asteraceae) family is nested in the order Astrales of Campanulids according to the APG III classification (The Angiosperm Phylogeny Group 2009). This family has 1700 genera's and largest number of accepted species 24,000 among angiosperm plant families and is distributed throughout the globe except Antarctica, Funk *et al*. (2009).

The Asteraceae are not only a large family abundant everywhere but, as may be expected, a diverse one. After the long outdated classifications of Bentham (1873) and Hoffmann (1890), the new classification of the Compositae by Bremer (1994) was the first approach at a modern phylogenetic analysis of this family. Bremer's classification was morphologically based, but with subsequently rapidly developing molecular data a new picture of the family phylogeny emerged, first summarized by Kadereit and Jeffrey (2007) and their associates. This modern approach culminated in the seminal and epoch-making work of Funk *et al.* (2009). At present, the basal groups of Compositae are recognized as subfamilies like Barnadesioideae, Mutisioideae, Hyalideae, Wunderlichieae, Carduoideae, Pertyoideae and Cichorioideae.

Hooker's Flora of British India of 1881 which is more than 134 years old. Since then a lot of ICBN nomenclatural changes have taken place and a large numbers of species which were

previously reported under the genus *Lactuca* have now been shifted to other genera such as *Dubaeya, Ixeris, Xeridium, Cicubita, Launaea, Sonchus, Mulgedium*, Bhellum and Singh(2015)

In India, there are about 177 genera and 1,052 species belonging to this family Rao *et al.* (1988). The family (exhibits, several type of morpho-anatomical characters, which are very helpful for the separation of this family from another families. One of the important characters of this family is the present of trichomes on the cypselar surface. Trichomes or hairs have long been of much importance in systematic investigation of angiosperms.

Anatomically, a trichome or hair is an epidermal outgrowth of diverse form, structure and function, Uphof (1962), Esau (1965), Fahn (1986, 1988). The hairs offer a rich field of investigation for a micro-morphologist because of their common occurrence and great diversity, simplicity in structure and easy availability for observation due to their superficial position, Sahu (1982 ,1983).

The types of hairs (epidermal outgrowths) have been reviewed and classified in early works, such as by De Bary (1884) who recognized four major categories, (i) Bladders, (ii) Hairs, (iii) Scales & (iv)Shaggy hairs. De Bary also noted that the presence of hairs in plant organs is a universal character in vascular plants. Later Cowan (1950) merged the 'Shaggy hairs' into the category of' hairs and various other categorizations of trichome type have been put forward. Similar approaches have been made in various plant families. For example, Goebel (1900) was the first to discuss the significance and relationships of trichomes in the tribe Rhinantheae of the family Scrophulariaceae.

One pioneer in the study of role and importance of hairs in taxonomy and their phylogenetic evolution was Solerader (1908) in his work entitled "Systematic Anatomy of the Dicotyledons". He again divided the hairs into two major categories (i) the clothing hairs and (ii) the glandular hairs. Solerader described the hairs present on the ovary of *Spilanthes oleracea* (now *Acmella oleracea*) as follows: "they consist of two long hair cells which are jointed together lengthwise along one side, but diverse apically so as to resemble swallows tail." Such types of hair are characteristic of the ovary or cypsela (achene) in the family Compositae Ramayya (1962) and these hairs were designated variously, 'Zwillingshaare' Kraus (1866), Hess (1938) , translated to 'twin hair's, Vaughan (1970), Grau (1977), Dietrich (1977) , or biseriate forked hair's, Narayana (1979), simply 'achenial hair's, Small (1919), Hess (1938) , Ramayya (1962,1963,1969,1972), Manilal (1963), Sahu (1976,1978,1979,1980,1982 ,1983,1984), Freire *et*

al. (2002), or sometimes 'duplex hair's, Macloskie (1883), Nichols (1893), Nordenstam (1977). Primarily a twin hair consists of two triangular or rectangular and usually short basal cells, completely or partly united with each other along their longitudinal walls, Sancho and Katinas (2002). Nordenstam (1968) described the twin hairs of Euryops as consisting of two parallel, short to elongate cells plus a smaller basal cell, three cells altogether.

Metcalfe and Chalk (1950, 1979, and 1989) have stated that trichomes possess taxonomic value, that both glandular & non-glandular hairs are present in the Compositae, and that the non-glandular hairs are of various morphological types, whereas the glandular hairs are more or less homogeneous with a uniform structure. Structures of hairs are greatly variable among different taxa. Spring (2000) has described more than 300 forms of plant trichomes. King and Robinson (1969, 1970, and 1979) have used the hair structure along with other epidermal features in determining many generic circumscriptions in the Compositae, especially in the Eupatorieae.

The detailed structure of trichomes from stem and leaves of 135 species belonging to 53 genera in tribe *Lactuceae* was studied by Krak and Mraz (2008). They recognized eight types and several subtypes, and they have focused on the utility of hairs in the infra tribal classification with the help of both light and scanning electron microscopy. Mukherjee (2012).

Diversity of leaf hairs and phylogenetic relationships on the basis of such hairs in different taxa of Artemisia has been discussed by Hayat *et al.* (2009) using both light microscopy and scanning electron microscopy. Eight major types of hairs were distinguished in their study.

The Asteraceae or Compositae are regarded as a fairly advanced family of the dicotyledons, Cronquist (1988), Takhtajan (1997). The classifications by Bentham (1873,1873), Cronquist, (1988), Takhtajan (1997). The classifications by Bentham (1873, 1873), Hoffmann (1890) and others have considered pappus as important character in sub familial, tribal and infra tribal classification.

In the classification of Bremer (1994), the traditional concept of two about equally large subfamilies, Asteloideae and Cichorioideae, was supplemented by a third, the smaller and geographically more restricted Barnadesioideae.

There are two views regarding the morphological nature of pappus. Initially, a non-calycine nature of pappus was advocated by Small (1919). This view was partially adopted by Sattler (1973), but has not been generally accepted by recent workers. The almost universally accepted view is that the pappus is calycine in nature, a modified calyx. This idea was introduced

many years ago by Lund (1872) and later it was taken up and confirmed by a number of workers, Philipson (1953), Carlquist (1957), Ramiah and Sayeeduddin (1958), Tiagi and Singh (1975) Cronquist (1977,1981).

Small (1919) recognized many types of pappus structure and in his opinion all are derived from the fundamental setose scabrid type. From the scabrid seta, he derived simple and plumose bristles, as well as paleaceous, aristate and coroniform pappus types, by fusion, splitting and reduction processes. Most denominations were adopted from early workers such as Berkhey (1761), Gaertner (1791) and Cassini (1826-1834).

It is generally considered that the pappus plays an important role in the dispersal of the one-seeded dry fruits, called achenes or cypselas. This is especially obvious in cypselas with numerous capillary 5 or plumose persistent pappus bristles, as in the common Dandelion, Taraxacum, and Ruderalia. The transportation in the air of papoose fruits is controlled by the relative humidity of the atmosphere as well as air current, Taliew (1895), Sheldon and Burrows (1973), Matlack (1987), Anderson(1992,1993). Not seldom, the pappus is caduceus (deciduous, fugacious) and is detached before dispersal of cypsela, although sometimes the ' pappus is inconsistently deciduous Shmida (1981). Already Bentham (1873) remarked that pappus was not always associated with the dispersal of cypsela.

The pappus is a taxonomically important and useful organ in Asteraceae, as noted by many synantherologists including Cassini (1827). In tribal and generic revisions and floristic work, descriptions of pappus are routinely given, although sometimes brief and superficial, and the work, descriptions of pappus are routinely given, infra generic taxa. An overview of the diversity in pappus structure within the subfamily Asteroideae was given by Mukherjee and Sarkar (2001).

It is generally considered that the Asteraceae is a natural family, similar to Brassicaceae, Poaceae and Apiaceae. The members of this family are easily recognized by some characteristic features which are pseudoanthial heads with a specialized type of pollen presentation mechanism and pappus structure, Mukherjee and Nordenstam (2008), nature of fruit, Das and Mukherjee,(2008) and their particular array of chemical weapons, Heywood *et al*, (1977).

Though, presence of pappus is an important character in this family, Mukherjee and Sarkar (2001), Mukherjee and Nordenstam (2008) but in some genera, pappus is absent. Some of the epappose genera are also endemic in some parts of the world as well the in India. The

information about as epappose genera and their distribution of identity has been obtained from Bremer (1994), Hajra *et al.* (1995), Kadereit and Jeffrey (2007), Mabberley (2008), Funk *et al.* (2009). Cass, Siegesbeckia, Hook, Arn and Dalzell (2013).

Structure and arrangement of pappus bristles or scales have played an important role for differentiation of taxa. On the basis of structure, or scales have played an important role for differentiation of taxa. The tip of the pappus bristle can be used taxonomically for the identification of the species in *Sonchus,* Mukherjee and Sarkar (1995).

One seeded indehiscent fruit of the Asteraceae is technically termed as cypsela, which is developed from inferior bicarpellary ovary. Fruit may or may not be associated with persistent calyx, usually in the form of either scales or bristles. As a result, this type of special fruit or cypsela is actually false fruit. The cypselar features are equally important like other floral and vegetative features for delimitation of taxa and for assessing their relationship. Thus a more extensive study of cypselar structure of some members of the tribe. Jana (2013)

Cypsela and pappus are two morphological features which are aiding in taxonomic classifications at tribal levels of Asteraceae, Talukdar (2008), Frangiote-Pallone and Antonio de Souza (2014), Talukdar and Mukherjee (2014). Cypsela and pappus morphology together with growth form, capitula size, florets, involucral bracts and leaf shapes were successfully used in separating daisy tribe Anthemideae into 12 sub-tribes, Bremer and Humphries (1993). For the taxonomic delimitation at tribe level, morphological diversity of cypsela has been used to distinguish the tribe *Heliantheae* and *Eupatorieae* of family Asteraceae from rest of the tribes by investigating carbonized cypselas, Bremer (1994),Talukdar T (2015).

The cypselar features are equally important like other floral and vegetative morphological features for delimitation of taxa and for assessing their relationship. Biswas, B K, Bar, and S K, (2014).

According to Haque and Godward (1984) the term 'carpopodium' was first used by Mattfeld (1923). Carpopodium is the basal abscission zone of the cypsela, which is attached to the receptacle and composed of one or more rows of cells, which are distinct morphologically from the rest of the cells of cypselar wall. Initial information about the different types of abscission zone of cypsela in Asteraceae was presented in the works of John (1921).

6. Research Gaps identified in the proposed field of investigation. (Based on the review)

In Rajasthan large number of publications dealing with the floral composition. The publication of flora Indian desert Bhandari (1978), Flora of south East Rajasthan (Sharma and Tiagi, (1979), Flora of Rajasthan (Shetty and Singh,1987,1991,1993). The flora of Rajasthan (Sharma 2002), the south and south east region (Tiagi and Aery,2007) and south central Rajasthan (Yadav and Meena 2011) added out knowledge of the floral composition of Rajasthan.

These studies will be of good significance in assessing the flora and ethno botanical study in southern Rajasthan, with particular family Asteraceae which has received sincere attention of botanical workers. No work has been done on the Asteraceae flora of Sothern Rajasthan except observation about some new taxa as genus *Soliva Ruiz* and Pavan, *Lactucaa seviola L.* and species *Bidens pilosa* L. var. minor (Bl.) Sherffy Meena (2014 a,b,c).

These studies will result in a valuable reference for all the students of taxonomy. Such type of work has never done in Rajasthan in any university. There is a huge gap with classical method of macro morphological study to micro morphological studies of family Asteraceae in Rajasthan. No gap is filled by any taxonomist in aspect of micro taxonomical, ecophysiological and ethno botanical studies definitely identifies some new aspects solves the problems regarding identification of Asteraceae flora of Rajasthan.

7. Major Hypothesis, if any

These studies will result in valuable reference for family Asteraceae in one way or the other in general and the student of Taxonomy and ethno botany. Also these will be useful to foresters, Medical scientists and those interested in biodiversity, traditional knowledge of indigenous people and conservation aspect of plants. The result of the study will also be useful in the revision of the state or national flora of India.

8. Research Methodology:

The study on of family Asteraceae from southern of Rajasthan will be based on the both extensive and intensive studies of the vegetation of area under study. Field survey will be carried out for collection of plants. Identification of plant species during field work will be done by compiling different floras available.

Floristic survey

The study area will be thoroughly surveyed in all seasons to procure maximum collections and their variants. The field work will consist of collection of plant specimens for herbarium, observation of the habit, habitat, phenology and distribution. The rainy season is perhaps the best time and attempts will be made all these years to visit as many places as possible during this favorable season. The other time suitable for collection is near about October-December when plants are in their full bloom. Besides these, the aquatic plants could be studied as by this time the turbulent water of the rainy season settle down and allow the aquatic vegetation to come up. Flowering stages of some trees also could be collected in this season.

Morphological studies

For morphological observation fresh as well as dried leaves, pappus and, flower will be studied in laboratory.

Macro-morphological studies of cypselas

In cases, where intact cypselas will be available, the first and foremost step will be to mark the posterior and anterior surface of the cypselas. Then 10 dry and 10 FAA preserved mature cypselas will be randomly taken in glass slides and graphed slides and observed under dissecting microscope (DM) and binocular microscope .Suitable images Will be taken using camera equipped microscope. Shape and direction of cypselas will be noted carefully. Length and width of the cypselas will be measured visually by graphed slides, in few cases they will be counted by ocular and stage micrometer. The length of the cypselas is defined as the length of the body of cypselas from basal meristematic zone (carpopodium) up to apical end excluding pappus. The width of the cypselas will be measured at the widest part of the cypselar body.

Micro-morphological studies of cypselas

Mature cypselas will be dipped in 1-5% NaOH solution for 2-7 days depending upon the hardness.Then they will be transferred into saturated chloral hydrate solution for few hours, repeatedly washed with water and properly stained in 0.2-0.5% aqueous Safranin solution. Suitable photographs will be taken using zoom digital camera .Nature of ribs, types, distribution and orientation of hairs, nature of surface cells, other epidermal structures and carpopodial cells will be critically observed. Pappus characters such as nature of pappus bristles, their number, arrangement, length and apex organization will also be examined

Ecophysiological studies

The phytochemical aspects will be carried out in the laboratory following various methods such as

Yem & Wills (1954) for Sugars

Plant extract (100 ml) will be taken in 25 ml test tubes and 6 ml anthrone reagent will be added, and then will be heated in boiling water bath for 10 min. The test tubes will be ice cooled for 10 min. The test tubes will be incubated for 20 minutes; at room temperature (25° C).Optical density will be read at 625 nm on a spectrophotometer. Blank will also be read in the same way. The soluble sugars will be calculated from the standard curve developed by using following the above mentioned method.

Bates et al. (1973) for proline :

The frozen plant material wiil be homogenized in 3% aqueous sulphosalicylic acid (0.01g/ 0.5 ml) and the residue will be removed by centrifugation at 12 000 g for 10 min. 1 ml of the homogenized tissue will reacts with 1 ml acid-ninhdrin and 1 ml of glacial acetic acid in a test tube for 1 hour at 100°C and the reaction will be terminated in an ice bath. The reaction mixture will be extracted with 2 ml toluene,will be mixed vigorously and will be left at room temperature for 30 min until separation of the two phases. The chromophore-containing toluene (1 ml, upper phase) will be warmed to room temperature and its optical density will be measured at 520 nm using toluene for a blank. The proline concentration will be determined from a standard curve using D-Proline.

Moore & Stein (1948) for amino acids

Total free amino acid content will be estimated according to Ninhydrin method given by Moore & Stein (1948). L Lysin will be used as standard amino acid to prepare standard curve.

Farkas and Kirlay (1962) for phenol

Total phenol will be isolated as per the method given by *Farkas and Kirlay (1962)*.Catachol at the concentration of 20 to 100 µg/ml will be used to prepare standard curve.To find out the stress tolerance of the plants effect of hyperthermia on cell membrane permeability and photosynthetic pigment will be worked out (Sullivan, 1967)

Extraction of Photosynthetic Pigment: Lichtenthaler and welburn (1983) method

Fresh leaves will be chopped into small pieces and exactly 0.1g of sample will be weighed and macerated in mortar and pastel with 2 ml of 80% acetone. The content will be centrifuged at 10000 rpm for 10 minutes. The residue will be re-extracted in 1 ml of 80% acetone for two times and centrifuged at 10000 rpm for 10 minutes. The supernatant will be pooled and made to 10 ml of 80% acetone. Approximately diluted extracts will be read on spectrophotometer for estimation of pigments.

Chlorophyll a (µg/ml) = 12.21(A 663) – 2.81(A646)

Chlorophyll b (µg/ml) = 20.13(A 646) – 5.03(A663)

Carotinoids (µg/ml) = (1000A 470 – 3.27[Chl a]-104 [Chl b])/227

Micro morphological features such as epidermal cell, stomata and trichomes especially of Asteraceae will be investigated by clearing the leaf with saturated chloral hydrate solution. Stripes of leaves will be taken from the fresh plant will be oxidized in Schulze's reagent (Potassium Chlorate and Cone. HNO_3) and heated over a spirit lamp for 2 - 3 minutes. The reagent will be decanted and the materials will be repeatedly washed with water. Then 1% aqueous solution of safranin will be added. The leaf cuticle will then be cleared using a fine brush and mounted in 70% glycerine. Stomatal index will be determined by using the formula:

$$I = S/S+E \times 100.$$

Where

I = Stomatal index

S = Number of Stomata

E = Number of epidermal cell.

Ethno botanical studies:

The undertaken work can be divided into three main stages:

(A) Field work in the tribal villages.

(B) Literature screening.

(C) Miscellaneous methods

Field work

Before proceeding on field visit the localities will be carefully selected on the basis of the available information of the area to be visited and the people to be studied, taking aid of maps, divisional forest working plans, floras, icons, literature on the tribes and obviously, discussions with relevant personnel. Data on climatic factors, tribal population, geographical conditions, land use pattern etc. will be obtained from district gazetteers, working plans of tehsil and other secondary sources. Information on various uses of plants will be collected through interview with local shepherds, medicine man and other knowledgeable elderly persons belonging to rural or tribal communities and herbal practitioners. Repeated enquiry on various applications of the same plant will be made from different persons of the same village and tribe to ascertain the correctness of information. The voucher specimens will be collected, processed as per herbarium methods identification will be done either in the field or in the laboratory following the different floras (Shetty and Singh 1987-1993); (Yadav and Meena, 2011) and will be deposited in the Herbarium, Department of Botany, M.L.V. Government College, Bhilwara, Rajasthan, India. The linguistic fluency, personality and social standing are crucial in establishment of rapport between the participants involved.

Ethnobotanical data will be collected first hand through:

I. Enquiry
II. Observations
III. Interviews
IV. Participation

Litreature screening

Our ancient literature will be tapped for information on medicinal plants. Rig Veda and Atharva-Veda which dates back to 2000 to 1000 BC, which are our oldest Vedic literature resources contain valuable information regarding medicinal plants of that period. Nearly 248 botanical drugs are mentioned mainly in Atharva-Veda and Rig-Veda. A glossary of medicinal plants have been mentioned in Charaka Samhita, Sushruta Samhita and Ashtanga Hridiyam.

Perhaps the outstanding example at least in modern times of the use of the literature is the huge compilation of all anti-tumor plants, cited in old texts and local folk medicine from all over the world for screening purpose at Cancer Chemotherapy National Service Centre (CCNSC).

Miscellaneous methods

Updating of nomenclature according to the rules laid down by "International Code of Botanical nomenclature (Vienna Code, 2005)", preparation of keys to genera and species and lastly the treatment of individual families. Names appearing in recent monographs, papers dealing with nomenclature in standard journal and latest floras will be adopted.

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Diversity of trichomes from mature cypselar surface of some taxa from the basal tribes of Compositae 2012.

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